UNIVERSITI PUTRA MALAYSIA

MOLECULAR CHARACTERIZATION OF COAGULASE POSITIVE STAPHYLOCOCCI ISOLATED FROM DOGS AND CATS

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FPV 2001 3

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By

OMER HASSAN MOHAMED HASSAN ARABI

Thesis Submitted in Fulfilment of the Requirement of the Degree of Doctor of Philosophy in the Faculty of Veterinary Medicine Universiti Putra Malaysia

September 2001

DEDICATION

TO MY LATE PARENTS AND ALL MEMBERS OF MY FAMILY
TO MY DEAR BROTHER OTHMAN
TO MY WIFE FIROZE AND MY DAUGHTER TWASUL

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

MOLECULAR CHARACTERIZATION OF COAGULASE POSITIVE STAPHYLOCOCCI ISOLATED FROM DOGS AND CATS

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OMER HASSAN MOHAMED

September 2001

Chairman: Abdul Rahim Abdul Mutalib, DVM, Ph.D.

Faculty: Veterinary Medicine

Isolation studies of coagulase-positive staphylococci were conducted on hospitalised and out patient dogs and cats, on three sites: skin, nose and ear, between January and September 1997. Three tests were used to differentiate between coagulase-positive staphylococci, namely: acetoin production, P agar supplemented with acriflavin and β-galactosidase test. Thirty-six *Staphylococcus aureus* and 90 *Staphylococcus intermedius* isolates were recovered from these animals. *Staphylococcus hyicus* was not isolated, the results indicated that the major coagulase-positive *Staphylococcus* species in dogs was *S. intermedius* and in cats was *S. aureus.*

The antimicrobial typing of *S. aureus* and *S. intermedius* was compared with the molecular typing methods such as: Polyacrylamide-gel Electrophoresis profiles of protein A concentration and whole cell proteins, and Polymerase Chain Reaction

(PCR)-based methods that include: Random Amplification of Polymorphic DNA (RAPD-PCR), Enterobacterial Repetitive Intergenic Consensus sequences (ERIC-PCR), Coagulase gene PCR amplification and Restriction Fragment Length Polymorphism (RFLP).

The antimicrobial typing differentiated *S. aureus* and *S. intermedius* isolates into 14 and 28 profiles respectively. Isolates of *S. aureus* and *S. intermedius* containing plasmids were 41.7% and 46% respectively. However, no correlation could be made between plasmid occurrence and antibiotic resistance profiles. The SDS-PAGE profiles of whole cell proteins grouped 24 *S. aureus* and 48 *S. intermedius* strains into 19 and 16 profiles respectively.

In PCR-based methods the isolates were typed using three primers. The combination of three primers for the RAPD gave 33 and 83 profiles of 36 *S. aureus* and 90 *S. intermedius* isolates respectively. ERIC primers grouped 24 *S. aureus* and 47 *S. intermedius* isolates into 19 and 43 profiles respectively. The coagulase gene from 24 *S. aureus* and 47 *S. intermedius* isolates showed limited discriminatory to the other methods and was least useful for the preliminary epidemiological studies. The restriction enzyme analysis of coagulase gene PCR products was very useful to increase the discriminatory power of coagulase gene PCR but required the use of multiple restriction enzymes. It was concluded that RAPD-PCR and ERIC-PCR are the best methods for typing *S. aureus* and *S. intermedius*.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENCIRIAN MOLEKUL STAFILOKOKUS POSITIF KOAGULASE DIPENCIL DARIPADA ANJING DAN KUCING

Oleh

OMER HASSAN MOHAMED

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Pengerusi: Abdul Rahim Abdul Mutalib, DVM, Ph.D.

Fakulti: Perubatan Veterinar

Kajian pemencilan stafilokokus-positif telah dijalankan di antara bulan Junuari dan September 1997, terhadap anjing dan kucing pesakit luar dan hospital, pada tiga tapak: kulit, hidung, dan telinga. Tiga ujian telah diguna untuk membeza di antara stafilokikus koagulase-positif iaitu, penghasilan asetone, ujian agar P ditambah akriflavin dan β-galaktosidase. Tiga puluh enam pencilan *Staphylococcus aureus* dan 90 pencilan *Staphylococcus intermedius* telah diperolehi daripada haiwan tersebut. Hasil kajian menunjukkan yang spesies *Staphylococcus* utama dalam anjing ialah *S. intermedius* dan dalam kucing *S. aureus*.

Pengetipan antimikrob *S. aureus* dan *S. intermedius* telah dibandingkan melalui kaedah pengetipan molekul seperti: profil Elektroforesis Poliakrilamida-gel protein dan protein A sel sepenuh, dan kaedah berasas Tindak Balas Rangkaian Polimerase (PCR): Penguatan Rawak DNA Polimorfik (RAPD-PCR), jujukan

Konsensus Antaragen Berulang Enterobakteria (ERIC-PCR), Penguatan PCR gen Koagulase dan Polimorfisme Panjang Fragmen Pengehadan (RFLP)

Pengetipan antibiogramer dapat membezakan pencilan *S. aureus* dan *S. intermedius* yang mengandungi plasmid masing-masing 41.7% dan 46%. Bagaimanapun tiada sebarang perkaitan berlaku di antara kewuiudan plasmid dengan profil ketahanan antibiotik. Profil SDS-PAGE protein sel sepenuh telah mengumupulkan 24 strain *S. aureus* dan 48 strain *S. intermedius* masing-masing kepada 19 dan 16 profil.

Dalam kaedah berasaskan PCR, strain boleh ditipkan dengan mengguna tiga primer. Gabungan tiga primer untuk RAPD memberikan 33 dan 83 profil masing-masing daripada 36 strain *S. aureus* dan 90 strain *S. intermedius*. Primer ERIC mengumpulkan 24 strain *S. aureus* dan 47 strain *S. intermedius* masing-masing kepada 19 dan 43 profil. Gen koagulase daripada 24 strain *S. aureus* dan 47 strain *S. intermedius* menunjukkan pembezaan terhad terhadap kaedah lain dan paling kurang kegunaannya dalam kajian epidemiologi awal. Analisis enzim pengehadan hasil PCR gen koagulase adalah paling tinggi kegunaannya untuk meningkatkan kuasa pembezaan PCR gen koagulase, tetapi ia memerlu penggunaan enzim pengehadan berbilang. Kesimpulannya ialah, RAPD-PCR dan ERIC-PCR merupakan kaedah paling baik untuk mengetip *S. aureus* dan *S. intermedius*.

ACKNOWLEDGEMENTS

All praise to Allah, the Merciful and the Benevolent. Had it not been due to His will and favour, the completion of this work would not have been possible.

I would like to express my sincere gratefulness and appreciation to my supervisor, Dr. Abdul Rahim Abdul Mutalib, who has devoted a lot of his time for invaluable guidance, advice, supervision and support throughout the course of this study.

I wish to express my sincere gratitude to the Dean of the faculty and my co-supervisor Professor Dato Dr. Sheikh Omar Abdul Rahman for his invaluable advice, support and continued encouragement towards the completion of this work and being helpful whenever I ran into difficulties.

Sincere thanks also to my co-supervisors, Associate Professor Dr. Saleha Abdul Aziz and Associate Professor Dr. Son Radu who have provided advice and helpful discussion that have enlightened and improved this study.

I would like to express my gratitude to Professor Dr. Abdul Rani Bahaman who had provided facilities of his laboratory during the course of this study.

I would like to thank the staff members of Veterinary Clinic and Hospital, Dr. Habibah Arshad, Dr. Vijayndra Madawar, Pn Halimah Abu Bakar and Pn Hasina Behgam. I wish to thank Mr Shahreer at the Laboratory animal's farm for his kind assistance. I deeply send my "Duaa" to our late helpful man Mr. Basri Kasim, I asked Allah to forgive him and ascend mercies to his grave and enlighten it for him. My deeply indebted thanks to Mr Hajariah Salamah that was very helpful and generous with his time. I am very grateful with Mr Fauzi Che Yosof for his skilled assistance during this study.

Also, I had been very fortunate in receiving assistance from a number of my colleagues and friends. They had to give up some of their valuable time to help me and it would not be possible to name all of them. However, I would like to thank Mr. Omer Hussabo, Dr. Izz Eldeen Babeker, Dr. Esam Mohamed Ali, Mr Elwaleed Awad, Mr. Eltahir Siddig, Mr Tarig Elsharif, Mr Mohamed Ghrisi, Mr Naseer Eldeen Elhadi, Pn Nemita Fefadrah, Mr Samuel Lihan, Pn Zunita Zakaria, Pn Siti Khirani and Ooi Wai Ling.

Lastly, I would like to thank my brother Othman Arabi for his unlimited support to me during my study. Grateful thanks to my supportive wife, Firoze Mustafa Suliman and my daughter Twasul who had to put up with my long absence from them during study, so that I could complete this work.

I certify that an Examination Committee met on 7th September 2001 to conduct the final examination of Omer Hassan Mohamed Hassan Arabi on his Doctor of Philosophy thesis entitled "Molecular Characterization of Coagulase Positive Staphylococci Isolated from Dogs and Cats" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the candidate be awarded the relevant degree. Members for the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy.

AINI IDERIS, Ph.D.
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Date:

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously submitted for any other degree at UPM or other institutions.

Omer Hassan Mohamed Hassan Arabi

Date: 7-11-2001

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LIST OF ABBREVIATIONS

Ab	Antibody
AP-PCR	Arbitrary primed polymerase chain reaction
Bp	Base pair
BSA	Bovine serum albumin
°C	Degree Celcius
cm	Centimetre
CNS	Central nervous system
CO_2	Carbon dioxide
D.W	Distilled water
D.D	De-ionised distilled
DNA	Deoxyribonucleic acid
dNTP	Deoxy-nucleotide triphosphate
Dr.	Doctor
e.g.	For example
EDTA	Ethylene diamine Tetra-acetate
ELISA	Enzyme Link Immunosorbent Assay
ERIC	Enterobacterial repetitive intergenic consensus sequences
g	Gram
G+C	Guanine + Cytisine
H_2O_2	Hydrogen Peroxide
h	Hour
hrs	Hours
HIS	Hyperimmune serum
i.e.	That is
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
Kbp	Kilobase pairs
KDa	Kilodalton
M	Molar
Mab	Monoclonal antibody
Mda	Megadalton
M.W.	Molecular weight
mg	Milligram
Min	Minute
Mins	Minutes
ml	Millilitre
mM	Millimole
nm	Nanometer
O.D.	Optical Density
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffer saline